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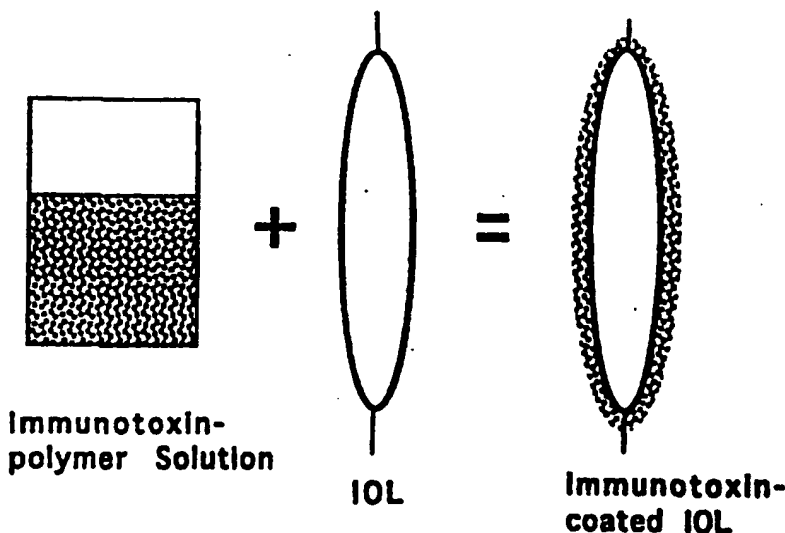
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(54) Title: POLYMERIC DEVICE FOR THE DELIVERY OF IMMUNOTOXINS FOR THE PREVENTION OF SECONDARY CATARACT

(57) Abstract

Intraocular devices together with methods for their preparation and use are provided, which are capable of sustained release of an antiproliferative agent, particularly an immunotoxin comprising a cytotoxic moiety. The intraocular devices are implanted in the eye where the immunotoxin is released from the intraocular device. The device may be used to inhibit proliferation of remnant lens epithelial cells, particularly after extracapsular cataract extraction. The components of the device can be provided as kits, but preferably are provided in a form suitable for use with an IOL or coated directly onto the surface of an IOL.

Immunotoxin-polymer Solution + IOL = Immunotoxin-coated IOL

POLYMERIC DEVICE FOR THE DELIVERY OF IMMUNOTOXINS
FOR THE PREVENTION OF SECONDARY CATARACT

INTRODUCTION

Field of the Invention

This invention relates to an intraocular polymeric device and methods for its preparation and use, wherein the polymeric device comprises a polymer and at least one cytotoxic agent, particularly an immunotoxin for prevention and treatment of secondary cataracts.

Background

An intact posterior lens capsule is required for implantation of a wide variety of intraocular lenses. A surgical technique that preserves the posterior lens capsule, and which has found use in the treatment of cataracts, is generically termed extracapsular cataract extraction. The technique includes extracapsular cataract extraction, phacoemulsification, endocapsular extraction and intercapsular extraction. Newer methods of extracapsular cataract extraction under development, such as the Kelman phaco fly, would remove a cataract through a puncture of the lens capsule and refill the lens capsule bag with a flexible substance or an inflatable lens to restore vision and to preserve or restore natural accommodation.

Extracapsular cataract extraction is a desirable method for removing cataracts due to a lower inci-dence of post-operative complications such as cystoid macular edema and possible retinal detachment. However, this surgical method is accompanied by a significant incidence of posterior lens capsule opacification, which may require additional surgical procedures such as posterior capsulotomy or repolishing of the posterior lens capsule to provide

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good vision. Virtually all pediatric patients and
approximately 50% of adult patients undergoing
extracapsular cataract extraction develop an opaque
secondary cataract within 3 to 5 years.

5   The pathogenesis of posterior lens capsule
opacification after extracapsular cataract extraction
is reported to be due to proliferation of remnant
lens epithelial cells on the posterior lens capsule
to form abortive lens "fibers" and "bladder" cells

10   (i.e., Elschnig's pearls). Various cytotoxic agents
are reported to inhibit this secondary cataract
formation or posterior lens capsule opacification,
including vincristine and vinblastine. Radiation has
also been tried and was reported to be promising.

15   Methotrexate and retinoic acid have been reported for
instillation in the anterior chamber of the eye to
kill residual lens epithelial cells and thus prevent
posterior lens capsule opacification. These methods
are relatively non-specific and can damage and/or

20   kill other cells in addition to the lens epithelial
cells.

        Techniques which are designed to more
specifically kill lens epithelial cells have included
introduction of immunotoxins capable of binding

25   specifically to residual lens epithelial cells on the
lens capsule remnant at the time of cataract surgery.
These immunotoxins have been shown to inhibit
proliferation of lens epithelial cells in vitro.
However, the immunotoxin activity is both dose and

30   time dependent and it is difficult to maintain the
immunotoxin in the posterior chamber at a
concentration and for a time sufficient to prevent
proliferation of and/or to kill the residual lens
epithelial cells. The immunotoxin passes through the

35   pupil into the anterior chamber where it is lost
through normal aqueous outflow. It would therefore
be of interest to develop methods and compositions

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for retarding loss of the immunotoxin from th
posterior chamber for use in preventing secondary
cataract formation or posterior lens capsule
opacification. Such a device would have the

5   advantage of providing a sufficient concentration of
immunotoxin in the vicinity of the eye for a time
sufficient to kill any lens epithelial cells
remaining in the eye after extracapsular extraction.

10   Relevant Literature

    Biodegradable microcapsules for use in the eye
are disclosed in Wang (U.S. Patent 4,853,224). An
eyeball medication dispensing tablet has been
disclosed in U.S. Patent 3,416,530. U.S. Patent

15   4,526,938 discloses copolymers which can be used to
provide for continuous release of polypeptides. See
also Heyrman, et al., (1989) J. Cataract Refract.
Surg., 15:169-175.
    Examples of polymeric delivery systems include

20   Sherwood, et al. (1992) Bio/Technology 10:1446-1449;
Hora, et al. (1990) Bio/Technology 8:755-757.
    Immunotoxins and their use for treating
secondary cataracts are described in U.S. Patents
4,871,350 and 4,432,751 and European Patent 0088606.

25   U.S. Patent No. 4,432,751 also discloses use of a
combination of monoclonal antibodies and complement
for preventing secondary cataracts. Complement-
fixing monoclonal antibodies which bind specifically
with lens epithelial cells and have low or no cross-

30   reactivity with other cells found in the anterior
segment of the eye are disclosed in U.S. Patent
5,202,252. The use of sugars such as trehalose to
prepare antibodies for storage is described in Rowser
(U.S. Patent 4,891,319).

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SUMMARY OF THE INVENTION

A novel intraocular device, together with
methods of preparation and use, is provided for
treatment and/or prevention of secondary cataracts.
5   The device comprises a biologically inert polymer and
at least one cytotoxic agent, particularly an
immunotoxin, capable of binding specifically to lens
epithelial cells. The cytotoxic agent is associated
reversibly with the polymer so as to provide for
10   sustained delivery of the cytotoxic agent following
implantation of the device into the eye. The
intraocular device is prepared in several ways
including by immersing a polymer matrix in a solution
of a cytotoxic agent whereby the cytotoxic agent is
15   pulled into the matrix by capillary action, by mixing
the cytotoxic agent with the polymer prior to
association with a support, such as an intraocular
lens (IOL), or by coating a polymer matrix bound
covalently or non-covalently to a support, such as an
20   IOL, with the cytotoxic agent. In use, the
intraocular device is introduced into the anterior or
posterior chamber of the eye during or following
cataract surgery. The cytotoxic agent is
continuously released from the intraocular device in
25   the vicinity of the posterior chamber at a
concentration and for a time sufficient to kill any
residual lens epithelial cells thus preventing the
formation of secondary cataracts.

30   ## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows preparation of an immunotoxin
coated IOL starting from a mixture of immunotoxin and
polymer in solution.

Figure 2 shows preparation of an immunotoxin
35   coated IOL using a two-step procedure: The polymer
is first coated onto the IOL, which is then
interacted with the immunotoxin solution. Upper view

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is IOL "edge" view; the intraocular device covers essentially the entire IOL surface. Lower view is IOL "face" view; the intraocular device essentially is a ring around the IOL.

5   Figure 3 shows the structure of a typical IgG antibody.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

The present invention relates to intraocular devices for preventing and/or treating secondary
10   cataracts, particularly at the time of or following extracapsular extraction. "Preventing secondary cataracts" is intended to mean preventing growth of any residual lens epithelial cells on the lens capsule remnant after removal of a primary cataract
15   by killing any residual lens epithelial cells present in the eye. The primary cataract can be of any type, including senile, juvenile and radiation-induced. "Treating secondary cataract" is intended to mean use of the device to inhibit proliferation of, preferably
20   to kill, lens epithelial cells which have grown across the optic axis of the posterior lens capsule following removal of the primary cataract.

Treating and/or preventing secondary cataract relies on the introduction of an intraocular device
25   into the posterior or anterior chamber of the eye, wherein the intraocular device comprises a polymer or macromolecule and at least one cytotoxic agent capable of selectively inhibiting proliferation of and/or killing any remnant lens epithelial cells
30   present in the posterior chamber. The cytotoxic agent is dispersed in the macromolecule, particularly reversibly associated to or physically entrapped in the polymer, where the polymer optionally is cross-linked to form a matrix as a means of decreasing the
35   rate of release of the cytotoxic agent from association with the macromolecule. In use, the device thus limits diffusion of the cytotoxic agent

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both temporally and spatially. The purpose of the described invention is to prolong the exposure time of any remnant of lens epithelial cells present in the posterior chamber to therapeutic concentrations

5   of cytotoxic agent.

The intraocular device may be provided in several forms. It may be provided as a stand-alone device which can be used alone or in conjunction with other devices such as IOLs. It may be provided

10 coated onto a support. It may be provided as components for bonding onto a support, for example, an IOL. Where the polymer itself is rigid enough to act as its own support, for example, crystalline, rubbery PEO-based hydrogels that are mechanically

15 strong but not brittle, the polymer may be cast into a form for use, in conjunction with an IOL, where the form of the intraocular device is, for example, a circular ("O") ring or a donut capable of fitting around an IOL or to fit in the lens capsule with an

20 IOL. The device is prepared having the diameter of the lens. It is then be placed in the eye so as to go around the periphery of the capsule. After the device is in place, an IOL can then be inserted.

Semi-rigid to flexible polymers which are

25 degradable by water, such as polyglycolic acid, polygalactin or polyglyconate may be used for preparing support structures such as O-rings. Once the structures are formed, they then may be treated or coated with the intraocular device, where the

30 polymer of the device may be the same or different from the polymer used to prepare the desired structure. Where the polymeric device is coated onto an IOL, generally it is coated onto the entire IOL, but more usually, the polymeric device is coated onto

35 the haptic portion or supporting loops of the IOL. Generally the IOL support is prepared from polymers such as polymethylmethacrylate or silicone.

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Various macromolecules may be used for preparing the intraocular device. Water soluble polymers may be used which may be mixed directly with the cytotoxic agent in solution. The water soluble
5   polymers may also be treated with cross-linking agents prior to interaction with a cytotoxic agent. Water insoluble polymers may also find use, where the polymer may be hydrated in a solution of the cytotoxic agent.

10   The characteristics of the polymer will include the following. The polymer must be biologically inert and physiologically compatible with tissues in the eye, i.e., it must be a polymer which does not in and of itself induce an inflammatory response or
15   affects the viability of cells in the eye tissue beyond that occurring as a result of the surgical procedure. For some applications it may be desirable to use a biodegradable polymer, i.e., one which is subject to hydrolysis or which is broken down by
20   biochemical processes such as enzymatic action. Examples of such polymers are the polymers used in dissolving sutures, for example, polyglycolic acid, collagen and the like.

One of the aims of this invention is to provide
25   a means for sustained release from the device of a cytotoxic agent in the posterior chamber of the eye, therefore the polymer should be one which provides for reversible association of the cytotoxic agent with the polymer. Polymers which provide this
30   characteristic include those capable of forming matrices so as to physically entrap the cytotoxic agent. Such a polymer may additionally be able to be cross-linked so as to provide a matrix of varying porosity. By varying the porosity, the rate of
35   diffusion of the cytotoxic agent may be varied. The polymer should be hydrophilic so that when it is dry it has the capacity to take up water and cytotoxic

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agent so as to form a hydrogel which finds particular
use in those cases where the polymer is to be formed
(cast) prior to interaction with the cytotoxic agent.
Generally, the polymer must not inactivate or react
5   with the cytotoxic agent, although in some
applications, a weak electrostatic charge on the
polymer may be useful for reversibly binding the
cytotoxic agent to the polymer. Examples of
compounds which may form gels upon hydration include
10   sodium hyaluronate and chondroitin sulfate.

The polymeric device may be prepared in several
ways depending upon the intended form and the
particular polymer to be used, as well as the nature
of the cytotoxic agent, e.g. whether it is a small
15   molecule or a large molecule, and the best way to
reverably associate the agent with the polymer. For
example, where the polymer is an insoluble polymer
the cytotoxic agent can be associated with a polymer
by hydrating the polymer in a solution of the
20   cytotoxic agent. The resulting hydrated polymer may
take, for example, the form of a gel or a more rigid
form. Examples of such hydrophilic polymers include
cross-linked polyethylene oxide (PEO) and other
polymers cross-linked with multifunctional alcohols
25   (polyethylene glycols) and diisocyanates.

Where it is desired to provide the intraocular
device with a support, particularly where the
combination of the polymer and the cytotoxic agent
has insufficient rigidity to cast into a form
30   suitable for use in the eye, the polymer,
particularly dry polymer, may be mixed with the
cytotoxic agent and coated onto a support material.
The support material may be any material which is
biocompatible with tissues in the eye such as, for
35   example, a ring of collag n or polyglycolic acid for
use with an IOL, or the intraocular device may be
coated directly onto an IOL. Providing the

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The preferred characteristics of the polymer
include capacity for noncovalently interacting with
or entrapping the cytotoxic agent. This capability
provides for a source of cytotoxic agent in the eye
5   which is released slowly into the eye and which
thereby serves to decrease degradation of the
immunotoxin which may occur from exposure to fluids
in the eye or to decrease the removal by normal
ocular processes of the cytotoxic agent from the eye.
10   The polymer may be soluble or insoluble and will be
capable of absorbing the cytotoxic agent either
chemically, e.g., by way of electrostatic charge, or
physically, e.g., by capillary action and/or
entrapment, without affecting its therapeutic
15   properties, while serving to retain the cytotoxic
agent at the site of introduction. The proteinaceous
materials may be a proteinaceous material that is
physiologically acceptable to the internal
environment of the eye of the host, generally a
20   human. The proteinaceous material may be an
individual peptide, but generally is a combination of
peptides or proteins, e.g., albumin or other
proteins, or structural proteins such as collagen,
where the protein or polypeptide is crosslinked to
25   provide for the desired matrix structure.
Where proteinaceous, particularly collagenous,
material is used for preparing the intraocular
device, it may be derived from any mammalian host
source, such as bovine, porcine or human, or may be
30   prepared, as available, by other techniques, e.g.,
recombinant DNA techniques. The collagen employed
may be natural collagen or may be modified, such as
tropocollagen, atropocollagen, or the like. The
collagen may be nonimmunogenic, immunogenic, or only
35   slightly immunogenic. Various methods for preparing
collagen or derivatives thereof in purified form for
administration to a mammalian host are known in the

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literature. These methods may be found in such
patents as U.S. Patent No. 3,949,073 and references
cited therein. Of interest is bovine collagen which
is purified and is obtained from young cows or
5   calves. Purification will normally involve
dispersion or precipitation from various media, e.g.,
dilute acetic acid.

In addition, the cytotoxic agents can be
employed encapsulated in liposomes or other
10   controlled rate release compositions, which are
included in the polymeric material, so as to provide
for separate and distinct rates of release of the
cytotoxic agents. In this way, multiphasic
compositions can be prepared, so as to provide for
15   sustained release of the cytotoxic agent over long
periods of time. Formation of liposomes with
inclusion of various materials is described in
Papahadjopoulos (1978) Annals of the N.Y. Academy of
Sciences, 308; Gregoriadis and Allison (1980)
20   Liposomes in Biological Systems (John Wiley and
Sons); Leserman et al., Nature (1981) 293:226-228;
Barhet et al., Supramol. Struct. Cell Bio. Chem.
(1981) 16:243-258; and Heath et al., Science (1980)
255:8015-8018.

25   When used with cytotoxic agents lacking
specificity for epithelial cells, the liposomes
themselves could be retained in the eye by, for
example, coupling the glycoprotein streptavidin to
the liposome as a biochemical linker. See, for
30   example, U.S. Patent 4,885,172. Monoclonal
antibodies or IgG capable of binding specifically to
epithelial cells can be coupled to the streptavidin.
Following introduction into the eye, the liposomes
bind specifically to lens epithelial cells. Other
35   methods of encapsulation also can be employed where
the cytotoxic agent is encapsulated in a

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biodegradable coat and the rate of release is related
to the thickness of the biodegradable coat.

The cytotoxic agent generally is a conjugate of
a protein macromolecule capable of binding at least
substantially specifically to epithelial cells,
particularly lens epithelial cells, as compared to
other cells which may be present in or in contact
with the anterior or posterior chamber of the eye.
For the most part, the cytotoxic compositions will be
conjugates of a monoclonal antibody or its equivalent
with a cytotoxic agent. However, where the
monoclonal antibody or its equivalent has the
capacity to fix complement, the monoclonal antibody
alone may be used. The monoclonal antibody may be
toxic by combination with either endogenous
complement, or as appropriate complement may be
injected subsequent to introduction of the monoclonal
antibody into the anterior or posterior chamber of
the eye.

The monoclonal antibody may be produced as a
result of hybridoma formation and expression by the
hybridoma, whether in culture or present as ascites,
a monoclonal antibody fragment, such as Fab, $F(ab')_2$,
Fv, a recombinant variable region, a T-cell receptor,
or the like. The monoclonal antibodies and receptors
may be any mammalian species, including murine,
rabbit, human or the like, or combinations thereof,
such as chimeric antibodies, having a human constant
region and a mouse or other mammalian source variable
region. The antibodies may be any class or subclass,
such as IgA, IgD, IgG, IgM, and may include IgG1, 2a,
2b, or 3 or the human equivalents thereof. The
monoclonal antibodies may be derived either from a
simple hybridoma cell line or may be a mixture or
"cocktail" of two or more monoclonal antibodies
derived from different hybridoma cell lines, where

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the antibodies would bind to the same or different
antigenic moieties as the epithelial cells.

The methods for preparing the monoclonal
antibodies are well established as evidenced by the
5   numerous references described above (see "Relevant
Literature"). An animal is hyperimmunized with a
suitable immunogen, with or without addition of
adjuvant. Various epithelial cells may be used as
the immunogen, particularly human epithelial cells,
10   including tumor cells originating from epithelial
cells, for example HeLa cells, although other species
may find use, e.g., primates. Whole cells are
preferred, however homogenates, membrane fragments or
the like, can be used. The source of the cells
15   includes cells in tissue culture, surgical specimens
such as tissues removed during cataract surgery,
biopsy specimens, and the like.

In accordance with the subject invention, a
mammal, which can be a mouse or other small mammal,
20   is hyperimmunized with the immunogen. Methods of
immunization are well known and are amply described
in the literature. The immunogenic material,
generally about 10^5 to about 10^6 cells or cell
equivalents, is injected with or without adjuvant
25   into the mammal, or by repeated injections over
relatively short periods of time. To ensure the
hyperimmunization of the animal, 2-6 subsequent
booster injections are administered. The animals are
then killed, usually within 1-5 days after the last
30   injection, when it has been determined that a
suitable titer of antibody has been obtained.
Antibody-producing cells such as spleen cells or
lymphocytes from the immunized animal are removed and
immortalized.

35   The method of cell fusion is not a critical
portion of this invention and various techniques may
be employed. Generally a nonanoic detergent, for

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example poly thyleneglycol (PEG), is used as the
fusigen. The antibody-producing cells, for example
spleen cells and myeloma cells are combined in the
presence of a known ionic detergent conveniently

5   PEG1540 and other additives, for example, serum-free
Dulbeccos modified Eagle's medium (SF-DMEM) for
approximately 5 minutes. The excess nonanoic
detergent is then rapidly removed by washing the
cells. The cells are promptly dispensed into small

10   culture wells at a relatively low density, ranging
from about 1×10^5/well to about 5×10^5/well in
appropriate medium, commonly a selective medium
comprising hypoxanthine, and aminopterin and
thymidine (HAT) medium.

15   After a sufficient period, usually one to two
weeks, colonies of hybrids are observed. The
colonies are then screened for antibodies which bind
at least substantially specifically to epithelial
cells. Once colonies producing the desired

20   antibodies have been identified, the colonies may be
perpetuated to provide for a continual source of the
desired antibodies.

To identify hybridomas of interest, antibodies
secreted by the immortalized cells are screened to

25   identify the clones that secrete antibodies of the
desired specificity. Screening of the hybridoma
clones may be by binding to epithelial cells,
particularly lens epithelial cells, using an ELISA
assay. Other screening methods include radioimmuno-

30   assay and immunohistochemical staining of cryostat
sections of ocular tissue. Furthermore, the
resulting monoclonal antibodies may be isolated and
modified by truncating the constant region by various
peptidase digestions. The monoclonal antibodies may

35   be reduced to provide for Fab fragments with
available mercaptan sites for conjugation to other

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compositions. T-cell receptors may be obtained as
described in WO85/03947.

To obtain hybridoma cell lines secreting
monoclonal antibodies directed to a single antigenic
determinant associated with epithelial cells, the
hybridoma cells may be cloned using, for example,
limiting dilution. The stage at which the cells may
be cloned is not critical to the invention, and may
be before identification of colonies secreting
antibodies of interest, or later. However, to avoid
overgrowth of antibody producing cells with non-
antibody producing cells, the colonies are preferably
cloned as soon after fusion as practicable.

For large-scale production of antibodies, the
hybridomas may be introduced into the peritoneal
cavity of a mouse or other mammal and grown as
ascites tumors. Antibodies may then be isolated from
the ascites fluid. Alternate methods for large-scale
production of monoclonal antibodies including
introducing subcutaneous tumors using the method
described and collecting blood from the animal. The
hybridoma cells can also be grown on a large-scale in
tissue culture. Where the cells secrete the
antibodies into the growth medium, the conditioned
growth medium containing the antibodies can be
collected for antibody isolation. Where the
hybridoma cells do not secrete the antibodies, the
cells may be collected, lysed using conventional
means, and antibody purified from the cell lysate.
Methods of purifying monoclonal antibodies are well
known to those skilled in the art.

The binding compositions having specificity for
epithelial cells, may be joined to a wide variety of
toxic agents which may be derived from microorganism
or plant sources. Of particular interest are the
toxic subunits of naturally occurring toxins, such as
ricin, abrin, diphtheria toxin, etc. See for example

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Oeltmann and Heath, _J. Biol. Chem._ (1979) _254_:1022-
1027; Yule and Neville Jr., _Proc. Natl. Acad. Sci._
USA (1980) _77_:5483-5486; Gilliland _et al._, _Proc._
Natl. Acad. Sci. USA (1978) _75_:5319-5323; U.S. Patent
No. 4,379,145; GB2034324 and Masuho _et al._, _Biochem._
Biophys. Res. Commun. (1979) _90_:320-326; and
Blythman, _Nature_ (1981) _290_:145, the relevant
disclosures of which are incorporated herein by
reference.

Illustrative toxin A-chains or similarly
effective moieties include diphtheria toxin A-chains,
enzymatically active proteolytic fragments from
Pseudomonas aeruginosa exotoxin-A, ricin A-chain,
abrin A-chain, modeccin A-chain, and proteins found
in various plants having similar activity, such as
the plants _Gelonium multiflorum_, _Phytolacca_
americana, _Croton tiglium_, _Jatropha curcas_, _Momordic_
charantia, wheat germ, the toxin saponin from
Saponaria officinalis (Thorpe _et al._, _J. Natl. Cancer_
Inst. (1985) _75_:151), Chinese cucumber toxin
Trichosanthin (McGrath et al., _Proc Nat Acad Sci_
(USA) (1989) _86_:2844-2848). Of particular interest
is ricin A-chain. Also, mutant species of the toxins
of the species may be used, such as CRM45 (Boquet _et_
_al._, _Proc. Natl. Acad. Sci. USA_ (1976) _73_:4449-4453).

The toxic agents and moiety providing for
binding to the epithelial cells may be linked,
usually by a bond which is cleavable cytoplasmically.
Convenient linkages include disulfide, particularly
where the toxic agent has an intrinsic sulfur, or
other links, such as peptide links, urea links,
thioethers, imines, amides, imides, amidines, etc.
Functional groups which may find employment include
carboxylic acid groups, amino groups, imines,
aldehydes, isocyanates, mercaptans, olefins, or the
like. In addition, more complex linking groups can
be employed, where a group may be bound to one of the

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moieties in the conjugate to provide for convenient
linkage to an intrinsic group of the other moiety.
For example, the N-hydroxysuccinimide ester of m-
maleimidoylbenzoic acid may be employed to prepare an
5   amide of the toxin, which may then be linked through
an available sulfur atom on the monoclonal antibody
to provide a thioether.

Exemplary cytotoxic agents may have the
following formula:

10                    $(AS_n)-(S-X-R)_m$

wherein:

AS_n indicates the toxic agent having one or more
sulfur groups as part of the agent; n is 1 or up to
the number of sulfur groups present in the toxic
15   agent which are present as available mercaptide
groups, generally being up to about 4; R is a
monoclonal antibody, T-cell receptor or derivative
thereof; and m is 1 up to n, usually being from 1 to
2; and X is a linking group and may be a bond or a
20   group of from about 1 to 20, usually 1 to 12 atoms
other than hydrogen, which include carbon, nitrogen,
oxygen and sulfur. Sulfur will normally be bonded to
carbon, particularly aliphatically saturated. X may
be aliphatic, alicyclic, aromatic, heterocyclic or
25   combinations thereof, generally having from 0 to 6,
more usually from about 0 to 4, preferably about 1 to
4 heteroatoms, wherein oxygen and sulfur are present
as oxo or non-oxo-carbonyl or the thio analogs
thereof, or ether (including thioether), and nitrogen
30   is present as amino or amido. For the most part the
heteroatoms will be bonded solely to carbon.

Illustrative groups linking the disulfide
include aminoethylene 3-propanyl methylene carbonyl,
α-succinimidyl, 3-propylenethiocarbonyl. The groups
35   which may be used are for the most part conventional
groups providing for a disulfide linkage. The
disulfide compound is one which is capable of

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reacting with the cell-specific ligand, whereby a
mercaptide group may be displaced from the disulfide,
resulting in a new disulfide linkage between the
toxic agent and the ligand.

5   For the most part, the linkages will be
aliphatic of from about 1 to 6 carbon atoms,
providing for an amide bond to the receptor, although
this is primarily a matter of convenience, not
necessary to the operability of the subject
10   compositions.

The cytotoxic agent can be prepared as a fusion
toxin by expression of a recombinant DNA sequence
encoding the cytotoxic agent. The required
recombinant DNA sequence is constructed by isolating
15   a macromolecule having the desired target cell
specificity (specificity domain) and ligating it to a
protein toxin gene (cytotoxic gene).

The specificity domain and cytotoxic domain in
general comprise portions of a single polypeptide
20   chain. The fusion toxin chain may be represented by
the formula:

$$Sp-Jp-Ct \qquad\qquad (1)$$
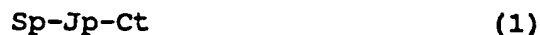
where Sp indicates the specificity domain; Jp
25   indicates at least one peptide bond, optionally
including a peptide sequence, joining the specificity
and cytotoxic domains; and Ct indicates the cytotoxic
domain, i.e., the peptide backbone of the specificity
domain is joined to the peptide backbone of the
30   cytotoxic domain through Jp. In its simplest form,
where Jp constitutes a single peptide bond, the
fusion toxin by the following structure:

$$X-NH-CHR_n-CO-NH-CHR_1-CO-Y \qquad\qquad (2)$$
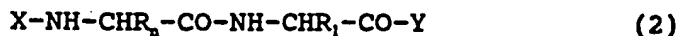
where $X-NH-CHR_n-CO-$ is the C-terminal nth amino acid
35   residue of domain X and $NH-CHR_1-CO-Y$ is the N-terminal
1st amino acid r sidue of domain Y. Alternatively,
Jp can be a joining peptide of arbitrary sequence and

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length which joins the peptide backbones of the
specificity and cytotoxic domains through the peptide
backbone of Jp, with the C-terminus of Jp joined by a
peptide bond to the N-terminus of domain Y and the
N-terminus of Jp joined by a peptide bond to the
C-terminus of domain X. Jp may be cleavable to allow
release of the toxin from the specificity domain. In
either case, the two domains are joined through at
least one peptide bond to make a single polypeptide
chain.

Although in many embodiments Sp comprises the
N-terminal end of the polypeptide, analogous to the
arrangement of natural antibodies, immunotoxins can
be created with Ct at the N-terminus. The
specificity and cytotoxic functions are mediated by
independent domains which in general are little
affected by the presence of the other. In rare cases
in which a particular fusion toxin functions
significantly less efficiently than the sum of the
constituent domains, steric hindrance is the probable
cause, and can be remedied by increasing the length
of the Jp or by reversing the order of the domains.

Fusion toxins are prepared by expression of a
recombinant DNA sequence encoding the polypeptide.
The required recombinant DNA sequence may be
constructed by the steps of: isolating cDNA encoding
the light and heavy chain genes of the monoclonal
antibody exhibiting the desired specificity for
epithelial cells from the corresponding hybridoma;
isolating cDNA encoding a desired protein toxin; iso-
lating the appropriate fragment of DNA from the heavy
and light chain genes; ligating the two V_H and V_L
encoding fragments in the same orientation and in-
frame through a DNA region encoding a joining peptide
as described in the definition of Jp to form the
specificity domain Sp; and ligating, in-frame, the
gene encoding the toxin to the gene encoding the

20.

specificity domain, optionally through a DNA encoding
a joining peptide described in the definition of Jp.

    An expression vector comprising the ligated DNA
is then constructed and used to transfect a host
5   organism which is then grown to express the fusion
toxin. If a secretory leader sequence has been
provided in the expression construct, the fusion
toxin can be isolated from the growth medium and
purified. Alternatively, where secretion is not
10   provided for, the cells can be lysed using methods
known to those skilled in the art and purified so as
to be substantially free of other cellular
components.

    In the first step for constructing the DNA
15   sequence encoding the specificity domain, a
monoclonal antibody capable of binding at least
substantially specifically to epithelial cells is
obtained as a result of conventional hybridoma
formation, expression, and screening, or as a result
20   of making antibody fragments using phage display
libraries (Huse et al., Science 246:1275, 1989;
Clackson et al., Nature 352:624-628, 1991; Soderlind
et al., Immunilogical reviews 130:109, 1992). The
monoclonal antibodies and receptors can be of any
25   mammalian species, including murine, rabbit, human or
the like. The antibodies can be of any class or
subclass, such as IgA, IgD, IgG, IgM, and can include
human subclasses IgG1, 2, 3, or 4, mouse IgG1, 2a,
2b, or 3, or the equivalents thereof in other
30   species; see Roitt et al., "*Immunology,*" (2nd ed.,
1989), Ch. 5. In preferred embodiments, most or even
all of the constant regions of the source antibody
are deleted from the fusion construct; consequently
the class and subclass of the source antibody are of
35   little importance.

    Methods for preparing monoclonal antibodies are
well established as evidenced by numerous references

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in the scientific literatur . An animal is
hyperimmunized with a suitable immunogen, with or
without addition of adjuvant. Various epithelial
cells can be used as the immunogen, particularly
5   human epithelial cells, including tumor cells
originating from epithelial cells, for example ME180
cells. Cells from other species also can be used,
e.g., primates. Whole cells are preferred, however
homogenates, membrane fragments or the like, can be
10   used. The source of the cells includes cells in
tissue culture, biopsy specimens, and the like.
Antibody-producing cells such as those from the
spleen of the immunized animal, are removed and
immortalized, typically by cell fusion with an
15   immortal cell line, to produce a hybridoma.
Alternative means of lymphocyte immortalization, such
as transformation with an immortalizing virus, e.g.
Epstein-Barr virus, are also possible. For
convenience, all immortalized monoclonal antibody
20   producing cells regardless of method of
immortalization are referred to hereinafter as
hybridomas.

To identify hybridomas producing monoclonal
antibodies of interest, antibodies secreted by the
25   immortalized cells, whether in culture or in ascites
fluid, are screened to identify the clones that
secrete antibodies of the desired characteristics.
The most important characteristic of such antibodies
is "substantially specific" binding to lens
30   epithelial cells, i.e., the ability of the antibody
to bind to lens epithelial cells at concentrations at
which its binding to other structures within the
posterior or anterior chambers of the eye is
negligible. Preferably the amount of antibody bound
35   per unit area to other cell types within the eye will
be less than 5%, more preferably less than 2%, of the
amount of antibody that is bound to lens epithelial

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cells. A second desirable characteristic of such
antibodies is high affinity binding to the epithelial
cell antigen. High affinity preferably means a
dissociation constant (K_d) of 10^{-7} M or less.

5   Screening of the hybridoma clones can be performed by
binding to epithelial cells, particularly lens
epithelial cells, using an ELISA assay. Other
screening methods include radioimmunoassay and
immunohistochemical staining of cryostat sections of

10   ocular tissue.

After a hybridoma producing a monoclonal
antibody having appropriate specificity for
epithelial cells is identified, cDNA encoding the
antibody is isolated from the corresponding

15   hybridoma. This is accomplished by preparing cDNA
from messenger RNA (mRNA) obtained from hybridoma
cells by conventional means, followed by ligation of
the cDNA into phage λ to produce an expression
library. The preferred vector is lambda ZAP II from

20   Stratagene (La Jolla, CA), although other variants
can be used. The ligation mixture is packaged by
conventional means and used to transfect *E. coli*.
The preferred strain of *E. coli* is XL1-Blue from
Stratagene, although other strains can also be used,

25   for example Y1090r- (*Molecular Cloning, A Laboratory
Manual*, Sambrook, J., et al., Cold Spring Harbor
Laboratory Press, Plainview, NY). The infected
bacteria are plated out on agar plates so that
isolated plaques are observed.

30   Expression of light and heavy chain antibody
genes is determined by immunoblot screening of the
plaques. The preferred antibodies used to screen the
λ library bind to the constant regions of either the
heavy or light chain of the mouse monoclonal antibody

35   and are conjugated to alkaline phosphatase or
peroxidase so that a color development reaction can
be used to detect the plaques to which they bind.

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Several plaques can be isolated to ensure that the
heavy chain and light chain genes are represented.
Those plaques which express antibody genes are
purified by reinfection followed by screening, as
5   described above, until a homogeneous preparation of
phage is obtained from each of the original plaques.

The homogenous λ lysogen culture is coinfected
with a helper phage, preferably M13KO7. The
coinfected culture yields M13 transducing phage
10   containing single stranded plasmid which harbors a
gene encoding either V_H or V_L.

The transducing phage containing lysate is
isolated by conventional methods and used to reinfect
a culture of XL1-Blue. These cultures are used to
15   stably maintain the cloned genes encoding the heavy
and light chain on a plasmid vector. These plasmids
are isolated and sequenced to confirm the identity of
the genes as described below.

The genes encoding the desired variable regions
20   of the light and heavy chains can also be cloned
using the polymerase chain reaction. Specific
primers are used in conjunction with a preparation of
cDNA isolated from the hybridoma to amplify a region
of the cDNA that encodes the V_H and V_L. These
25   amplified DNA fragments are then cloned into plasmid
vectors

The structure of a typical IgG antibody is shown
in schematic form in Figure 3 with the variable
regions of the heavy and light chains as indicated.
30   The variable regions in turn contain alternating
framework (FR) and complementarity-determining (CDR)
regions. In each chain, four FRs bracket three CDRs.
The CDRs provide the surfaces which interact directly
with antigen, while the FRs position the CDRs ad-
35   jacent to each other in proper tertiary conformation.
CDRs have variable sequences for antibodies of

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different specificities, whereas the FRs are more
conserved for a given antibody type and species.

Primers for PCR amplification of the heavy and
light chain variable regions are chosen to bind to
5   the 5' and 3' ends of the respective variable
regions. Primers for the 3' ends of the sequence
encoding the light chain and heavy chain variable
regions (V_L and V_H, respectively) are readily obtained
from the sequences encoding adjacent light chain and
10   heavy chain constant domains.

The 5' primers can be obtained in several ways.
First, the heavy and light chain N-termini of a
particular monoclonal antibody having the desired
characteristics are partially sequenced for 6-10
15   amino acids, and a DNA primer is synthesized based
upon the corresponding deduced nucleotide
sequence(s). Alternatively, a primer is designed
corresponding to relatively conserved leader
sequences or first framework region (FR_1) sequences of
20   the heavy and light chains characteristic of the
hybridoma source species.

The appropriate sets of primers are used to
amplify the cDNAs which encode the variable regions
of the light and heavy chains (See Chaudhury (1989);
25   Pluckthun, *Bio/Technology* (1991) 9:545; Larrick et
al., *Bio/Technology* (1989) 7:934.) The amplified V_L
and V_H DNA fragments are purified by conventional
techniques such as agarose gel electrophoresis on low
melting agarose and cloned into plasmid vectors.
30   Several copies, generally at least three
independently isolated copies, of the variable region
heavy and light chain encoding DNA fragments
sequenced to ensure that the variable regions contain
the expected three complementarity determining
35   regions (CDR1, CDR2, and CDR3) bracketed by f ur
framework regions (FR1, FR2, FR3, and FR4). The
variable framework regions can be abbreviated or

25.

modified, including humanized. Isolated heavy or
light chain variable domains have selective binding
affinity for their target antigens even without the
presence of the complementary chain. (See Ward,
5   *Nature* (1989) <u>341</u>:544.)

Although V_L and V_H peptides are capable of
binding independently to antigen and either could be
used alone as the specificity domain, the combination
of both creates a single chain specificity domain of
10   greater affinity and selectivity and is preferred.
Appropriate DNA sequences encoding the V_L and V_H are
joined in-frame, preferably through a DNA sequence
encoding a "linker" region comprising one or more
peptide linkages, to make a DNA sequence encoding a
15   single chain specificity domain. Necessarily the
single polypeptide produced by expression of the
above DNA coding region has its composite domains
joined through peptide linkages or bonds.
Specificity domains of this structure will correspond
20   to the general formula:

$$V_H'-Li-V_L' (3)$$

where Li indicates the linker region and V_L' and V_H'
indicate light and heavy chain variable domains,
respectively; the primes indicate that the variable
25   regions can be substantially modified from the
sequence of the parent antibody, especially in the
FRs.

Either V_L or V_H can provide the N-terminus of the
epithelial cell specific antigen binding or
30   "specificity" domain. The intervening linker region
can be of arbitrary length and sequence, but should
be of sufficient length to permit the V_L and V_H
polypeptides to interact correctly and provide for an
appropriate tertiary structure. The distance between
35   the C-terminus of V_L and th N terminus of V_H has been
reported to span 3.7 nm; *see* Whitlow and Filpula,
"Single Chain F_V Proteins and/or Fusion Proteins," in

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Methods: A Companion to Methods in Enzymology (1991).
Linkers of 10-30 amino acid residues are preferr d,
with those of 14-25 amino acid residues being more
preferable. Flexible linkers also are preferred;
these may be designed using small soluble amino acids
such as alternating glycine and serine residues. At
a few positions along the linker, charged residues
also can be introduced to enhance water solubility of
the linker, since the N-terminal and C-terminal ends
of the variable regions to be linked in general are
solvent accessible. *See* Chaudhury, *supra*; Sastry et
al., *Proc. Natl. Acad. Sci. USA* (1989) *86*:5728-5732.

The specificity domain is cloned into an
expression vector to permit testing of the resulting
polypeptide for binding affinity and specificity.
Expression of functional specificity domains is
accomplished from bacterial hosts such as *E. coli*, or
from eukaryotic hosts including yeasts, insect cells,
or mammalian cells. Naturally, an appropriate
expression vector is matched to the anticipated host
cells. An appropriate expression vector comprises a
promoter capable of providing transcription in the
host species, transcriptional and translational start
sequences, optionally a signal sequence to provide
for secretion, the V_L and V_H coding sequences joined
by a linker region, and translation and transcription
termination sequences. Optionally, DNA encoding
portions of the heavy and light chain constant
regions may be included at the 3' ends of the V_L
and/or V_H coding regions. The resulting expression
vector is used to transform the appropriate host cell
and obtain expression of the specificity domain. The
expressed epithelial cell binding or specificity
domain polypeptides are then screened to identify
clones exhibiting lens epithelial cell specific
binding. Screening for the specificity domain
polypeptides is performed essentially as described

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supra for monoclonal antibodies. It generally is performed by radioimmunoassay or by competition with parent monoclonal antibody binding in an ELISA assay.

The specificity domains of polypeptides of the present invention are not restricted to domain sequences which are identical to those of the source monoclonal antibodies. In particular, the term "specificity domain" of the present invention encompasses an epithelial cell binding domain which is homologous to the variable region of an antibody heavy or light chain.

Other toxic agents may also be used, such as bismuth non-diffusively linked to the monoclonal antibodies or receptors as described by Waldman, _J. Amer. Med. Assoc_.

Alternatively, liposomes may be linked to the monoclonal antibodies or receptors, where the liposomes contain various cytotoxic agents, such as methotrexate, 5-fluorouracil, any of the above toxins, or the like. See, for example, Szoka and Papahadjopoulos, _Proc. Natl. Acad. Sci. USA_ (1978) 75:4194-4198 and Szoka _et al._, _Biochem. et Biophys. Acta._ (1980) 601:559-571 for the preparation of liposomes, which disclosures are incorporated herein by reference. Linking of antibodies to the liposomes has been amply described in the literature, see for example Heath _et al._, _Proc. Natl. Acad. Sci. USA_ (1983) 80:1377-1381 and Leserman _et al._, _Nature_ (1981) 293:226-228, which disclosures are incorporated herein by reference.

Other cytotoxic agents conjugated to the binding moiety may also be employed in conjunction with the process of this invention. The cytotoxic agent can be used with other agents such as lysomotropic amines, carboxylic ionophores and calmodulin inhibitors. Examples include ammonium chloride, methylamine, chloroquine, monensin or verapamil. For

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descriptions of th se agents, see, for example, Casellas et al., _Immunotoxin_ (1988) Kluwer Academic Publishers; Myers et al., _Blood_ (1984) 63:1178-1184; Casellas, _J. Biol. Chem._ (1984) 259:9359-9364; and Akiyama, _J. Cell Physiol._ (1984) 120:271-279.

The intraocular device comprising the polymer and the cytotoxic agent may be used _in vivo_, by introduction directly into the posterior chamber of the eye so as to provide for a higher concentration of the immunotoxin in the immediate vicinity of the posterior chamber and the lens capsule and remnant lens epithelial cells than in surrounding structures. The amount of the immunotoxin released from the intraocular device in a given period of time can be varied, depending upon the total amount of cytotoxic agent associated with the intraocular device and by the composition of the polymer, for example, the concentration of polymer, the viscosity, and the like.

For example, to decrease the rate of release, the polymer may be cross-linked so that diffusion is controlled by the size of the cytotoxic agent, or the polymer may contain a slight charge, so that the attraction between the polymer and the cytotoxic agent is more than simple diffusion into the polymer but does not constitute covalent bonding to the polymer.

The amount of cytotoxic agent associated with the intraocular device is of an amount sufficient to kill at least substantially all of the remnant lens epithelial cells in the eye following release from the device. Generally a total amount of between 0.1 to 20 µg, more usually 0.5 to 10 µg, preferably 1.0 to 5 µg is associated with the intraocular device. The amount of release of immunotoxin from the intraocular device is usually from about 10-25% for the initial hour of release, and reaches a steady

state of amount of release of about 10% to 20% per
hour thereafter.

5   The intraocular device may comprise a single
cytotoxic agent reversibly associated with a polymer
or it may comprise more than one cytotoxic agent.
Other cytotoxic agents and/or antiproliferative drugs
which may be used include methotrexate and
daunorubicin. See, for example, Shawler et al., _J._
Biol. Resp. Mod. (1988) _7_:608-618; Dillman et al.,
10   _Cancer Res._ (1988) _48_:6097-6102. To provide the
necessary specificity of action, the cytotoxic agents
are preferably bound to an antibody, generally a
monoclonal antibody, capable of binding at least
substantially specifically to epithelial cells.
15   The intraocular device can be prepared by
combining the various components in a sterile
environment. The polymer and the cytotoxic agent can
be provided in a convenient form, usually in the form
of a solution, although in some instances the polymer
20   may be provided as a solid, which is then coated with
the cytotoxic agent directly, or which is coated with
a polymer/cytotoxic agent dispersion. Preferably,
the intraocular device is provided with an IOL
support, the intraocular device being coated onto the
25   surface of the lens so as to provide for ease of
introduction into the posterior chamber or lens
capsule. The intraocular device either with or
without an IOL support, should fit inside the lens
capsule for fixation in the ciliary sulcus and/or
30   other parts of the eye wall with or without sutures.
The intraocular device could also be placed in the
anterior chamber when there is no or little lens
capsule left, so that the immunotoxin could diffuse
into the posterior chamber.
35   When the polym r comprises collagen or a
derivative thereof, the collagenous material will
normally be provided as a uniform dispersion of

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collagen fibers in solution. The cytotoxic agent may
then be added to the collagenous dispersion with
agitation to ensure the uniform dispersion of the
cytotoxic agent in the resulting mixture. The amount
of cytotoxic agent associated with the polymer will
be sufficient to substantially completely or
completely kill all of the lens epithelial cells
following release from the polymer. Other materials,
as appropriate, may be added concomitantly or
sequentially. After ensuring the uniform dispersion
of the various components in the mixture, the mixture
may be sterilized by any means which will not
interfere with the biological activity of the
antiproliferative or other agents and sealed in an
appropriate container. Preferably, sterilization
will be achieved by using aseptic techniques and
aseptic conditions to admix already sterile
components.

In addition to the major components, a number of
minor components may also be included for a variety
of purposes. These agents will for the most part
impart properties which protect the stability of the
composition, control the pH, or the like, such as
stabilizers including sugars, wetting agents, etc.
Illustrative agents include phosphate or acetate
buffers, methyl or propylparaben, polyethylene
glycols, etc. These agents generally will be present
in less than about 2 weight percent of the total
composition, usually less than about 1 weight
percent, and individually may vary from about 0.001
weight percent to about 1 weight percent.

As already indicated, in some instances the
immunotoxin will be encapsulated, particularly in
liposomes. Liposomes are prepared from a variety of
lamellar-forming lipids including phospholipids,
e.g., phosphatidylcholine, phosphatidylethanolamine,

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etc., gangliosides, sphingomyelins, steroids, .g., cholesterol, etc.

Where the cytotoxic agent is a combination of monoclonal antibody and complement (either endogenous and/or exogenous), the monoclonal antibody can be introduced as described above for the cytotoxic agent. If exogenous complement is to be used, it is introduced, generally after 30 to 60 mins., into the anterior chamber in an amount of from 25-200, more usually from about 50-150 μl which agent will be present in an amount sufficient to substantially completely or completely kill all of the lens epithelial cells. The complement is a standard complement. By complement is intended normal serum of men or other vertebrates which comprises about 9 major proteins which react with antigen-antibody complexes to cause damage to cell membranes, and including lysis. A complement is usually supplied as serum, for example, rabbit serum. A typical complement and its preparation useful in the present invention is described in Monoclonal Antibodies (1980), Printing Press, New York Eds. Kennett, et al. pp. 391-392 which publication is incorporated herein by reference. Alternatively, it is possible to use patient complement activity which may be obtained in sufficient concentration from aqueous humor during surgery. The complement is prepared in an appropriate dilution of rabbit serum in a physiologically acceptable medium containing Mg^{2+} and Ca^{2+}.

Generally, the cytotoxic effect will be realized within a relatively short time after binding of the cytotoxic agent to the cells, generally 0.5-15 hrs, depending upon the concentration of cytotoxic agent used and the rate of release of the cytotoxic agent from the intraocular device. Binding itself will occur within a relatively short time, after the

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instillation of the intraocular device. Where the
cytotoxic effect is realized by the combination of
monoclonal antibody and complement, the cytolytic
effect is realized within a relatively short time

5   after the introduction of complement, usually in
about 0.5 hr. - 1.0 hr; lysis of the target cells can
be used to evaluate the onset of the cytolytic
effect.

The development of secondary cataract can take

10   from a few months to several years. Clinically, a
secondary cataract is determined by slit lamp
microscopy presenting as the appearance of lens
epithelial cells growing on the posterior lens
capsule. The invention described herein would allow

15   for the prolonged release of therapeutic
concentrations of lens epithelial cell specific
cytotoxic agents that would destroy these cells and
hence prevent their proliferation. Thus, slit lamp
microscopy can be used to determine that development

20   of a secondary cataract has not occurred. In cases
where a secondary cataract does occur, either
subsequent to treatment with the intraocular device,
or in the absence of treatment, the intraocular
device may be introduced into the anterior or

25   posterior chamber as previously described for use at
the time of cataract surgery for removal of a primary
cataract.

The IOL may be provided already treated with the
polymer in a form ready for reaction with the

30   immunotoxin or as an IOL or other support coated with
polymer and immunotoxin. Alternatively, the
components for preparing the intraocular device are
provided as lyophilized powders for reconstitution,
as concentrated solutions, or ready for use. The

35   subject compositions can be provided as kits for use
in one or more operations. The kits will include the
polymer and the cytotoxic agent in separate

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containers, either as concentrates (including
ly philized compositions), which may be further
diluted prior to use in an appropriate diluent for
use in the eye or at the concentration of use, where
5   the containers can include one or more dosages.
Conveniently, single dosages may be provided in
sterile vials, so that the physician may employ the
vials directly, where the vials have the desired
amount and concentration of agents. Thus, the kit
10   may have a plurality of vials containing the polymer
as well as the cytotoxic agent in appropriate
proportional amounts. Where the containers contain
the formulation for direct use, usually there will be
no need for other reagents for use with the method,
15   although optionally the kit may include separate
containers of complement for use with the IOL.

The following examples are offered by way of
illustration and not by way of limitation.

20   EXPERIMENTAL
Preparation of monoclonal antibodies capable of
binding at least substantially with lens epithelial
cells and methods for preparing immunotoxins are
provided for example in U.S. Patent No. 4,871,350 and
25   references cited therein, which disclosures are
hereby incorporated by reference. Monoclonal
antibody-producing cell lines have been deposited
with the ATCC Accession Number HB9747.

30   Example 1
Effect of polyoxypropylene - polyoxyethylene
block polymer (Pluronic® F68) on
antibody binding to ME180 cells
Monoclonal antibody 4197X at 2 mg/ml was mixed
35   with F68 to btain a final concentration of antibody
of 1 mg/ml and 10, 5, 2, 1 and 0% F68. These
solutions were incubated for 1 hr at 37°C followed by

34.

24 hrs at 4°C. Aliquots (100 µl) were removed after
the 1 and 24 hr time points, and diluted to a final
concentration of 10 µg/ml antibody in DMEM containing
10% FBS and a standard amount of ^{125}I-4197X.

5   ME180 cells (human cervix epithelial obtained
from the ATCC) were grown to confluence in 96-well
plates. The diluted antibody (200 µl) was then added
to the culture plates containing the ME180 cells and
evaluated for its capacity to competitively inhibit
10   the binding of ^{125}I-4197X to the ME180 cells. The
wells were incubated for 1 hr at 37°C after which
they were washed to remove unbound antibody. To
determine cell-associated radioactivity, the cells
were solubilized in 10% SDS (50 µl per well) and the
15   soluble extract harvested with a cotton-tipped
applicator stick which was then counted in a gamma
counter. Binding was compared to the cell-associated
radioactivity in the absence of competing antibody.
Preincubation of antibody with F68 had little effect
20   on its subsequent ability to bind to target cells.

Example 2
Determination of volume of F68 solution
associated with an intraocular device (IOL)
25   Two solutions of F68 and ^{125}I-4197X antibody
were prepared. One preparation contained 4197X-RA
immunotoxin at a concentration of 50 µg/ml. The
second preparation contained an equivalent volume of
buffer instead of immunotoxin. The radioactivity in
30   1.0 ul of the immunotoxin preparation was determined
to be 13,501 cpm. IOLs were immersed in these
solutions and excess liquid allowed to drain off.
The radioactivity associated with one of the IOLs was
48,438 cpm. The volume of F68 immunotoxin mixture
35   associated with the IOLs was calculated to be 3.6 µl.

35.

Example 3
Evaluation of Release and Cytotoxicity
of Immunotoxin from Intraocular Device
Coated onto an IOL

5   The release of radioactivity from an intraocular
device coated onto an IOL, to cell culture medium is
shown in Table 1. Two solutions of F68, immunotoxin
and ^{125}I-antibody were prepared, as described above.
One preparation contained immunotoxin at a final
10   concentration of 50 ug/ml. The final concentration
of F68 was 10%. The other preparation contained an
equivalent volume of buffer instead of immunotoxin.
IOLs (sterilized by autoclaving) were immersed into
these solutions and excess liquid allowed to drain
15   off. The IOLs were allowed to dry for 2 hours at
room temperature (until tacky). The IOLs were then
placed in cell culture wells (24 well plate)
containing ME 180 cells (25% confluent) and 1.0 ml of
cell culture media containing 10% fetal bovine serum.
20   The cells were incubated (37°C, 5% CO_2) until control
wells reached confluence. At various times during
incubation, samples of culture supernatant (50 ul)
were removed and counted for radioactivity. At the
end of the incubation period, the wells were examined
25   microscopically for cell viability. Photographs were
taken of all wells for later verification of results.

Viable cells were present in all wells not
containing immunotoxin. IOLs alone had no effect on
cell growth. There was a marked lack of cell growth
30   and cell viability in wells containing immunotoxin.
At 0.175 ug immunotoxin/IOL in 1.0 ml of cell culture
media and assuming all immunotoxin was released into
the media the effective concentration would be 0.175
ug/ml. The IC_{50} of immunotoxin alone for continuous
35   xposure as used in this experiment is approximately
0.02 μg/ml.

36.

Table 1
Release of [125]I-4197X from Intraocular Device
(Polymer = F68) Coated onto IOL Support

Time[1]	Total Released Radioactivity
	(cpm/well)
0	0
1	22,896
3	35,485
18	59,128
72	62,223

---

[1] Hours after introduction of IOL into cell culture.

Example 4
Determination of volume of F68 solution
and amount of immunotoxin associated with
a intraocular device after drying

Three solutions of F68, immunotoxin and [125]I-
immunotoxin ([125]IT) were prepared. The radioactivity
in a specified volume (cpm/10ul) of each preparation
was determined. The three preparations had a final
concentration of 10% F68 and 0, 4 and 20 ug/ml of
immunotoxin. The final concentration of [125]I-
immunotoxin was the same in all three solutions and
determined to average 1,434 cpm/μl. The immunotoxin
concentration of the labeled material was very low
and did not significantly contribute to the overall
immunotoxin concentration. IOLs were immersed (three
times at 2 minute intervals) in these solutions and
excess liquid allowed to drain off. The volume of
F68 associated with the IOLs was determined by
counting three of the IOLs in a gamma counter. The
results were an average of 8,915 cpm ± 1812 cpm/IOL.
Using this number, it was calculat d that IOLs
immersed in 4 ug/ml immunotoxin had 0.025 ug of

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associated immunotoxin/IOL and IOLs immersed in 20
ug/ml immunotoxin had 0.124 ug of associated
immunotoxin/IOL.

5   Example 5
                  Effect of Immunotoxin released from
                   intraocular devices on human lens
                         epithelial cells in vitro
                The release of radioactivity from IOLs coated
10   with the intraocular device to cell culture medium,
        is shown in Table 2 (below). Three solutions of F68,
        immunotoxin and ^{125}I-antibody was prepared as
        described above. IOLs (sterilized by autoclaving)
        were immersed into this solution and excess liquid
15   allowed to drain off. The IOLs were allowed to dry
        for 18 hours until dry. The IOLs were placed in cell
        culture wells (24 well plate) containing HLE cells
        (from cataract surgery capsule explants and
        maintained under tissue culture conditions) in 1.5 ml
20   of cell culture medium. The cells were incubated for
        7 days. At various times (15 min., 60 min. and 24
        hrs.), samples of culture supernatant (50 ul) was
        removed and counted. At the end of the incubation
        period, wells were examined microscopically for cell
25   viability. Photographs were taken of all wells. At
        the end of the observation period IOLs were removed,
        rinsed once in PBS, and counted. As indicated in
        Example 4, the initial average IOL-associated cpm was
        8,915 ± 1,812. Following incubation in cell culture
30   medium, the average cpm associated with the IOL was
        153 cpm which corresponds to a release of 98.3% of
        the radioactivity associated with the intraocular
        device.
                Viable human lens cells were present in all
35   wells not containing immunotoxin. IOLs alone had no
        effect n cell growth. There was a mark d lack of
        cell growth and cell viability in wells containing

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immunotoxin. Cells exposed to IOLs coated with
immunotoxin at 4 ug/ml (0.025 µg/IOL) were partially
affected. No viable cells were present in wells
containing IOLs coated with immunotoxin at 20 ug/ml
(0.124 µg/IOL).

Table 2

Release of [125]I-4197X-RA from

Intraocular Device (polymer = F68)

Coated onto an IOL into Culture Supernatant

Time	Mean Total Released Radioactivity[1,2]/Well	
	in cpm	% Initial IOL associated radioactivity
15 minutes	3,360	38
60 minutes	4,590	51
24 hours	10,890	122

[1]Control = 297 cpm/IOL

[2]Initial IOL-associated radioactivity before
being placed into cell culture = 8,915 ± 1,812
cpm/IOL

Example 6

Examination of Various Polymers for Coating

IOLs with Immunotoxin

Plastic culture wells were used to simulate
coating of IOLs. Immunotoxin (4197X-RA) was mixed
with various polymers (See Table 3) and examined for
cytotoxic activity following drying on to the plastic
tissue culture wells. The simulated IOLs were
prepared as follows. Polymer stock solutions were
prepared in phosphate buffered saline (PBS) and
sterilized by autoclaving. Equal portions of polymer
and sterile immunotoxin (at 770 ug/ml) or polymer and
PBS were mixed under sterile conditions and 20 ul of
each of the resulting mixtures added to individual

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wells of a 24 well cell culture dish. The solutions were allowed to dry in a sterile hood with lids open at room temperature for 3 hours.

1.0 ml of cell culture media (M199 containing 10% fetal bovine serum) was added to simulated IOL to rehydrate the immunotoxin followed by gentle agitation for 30 minutes at room temperature. Following rehydration individual supernatants developed from simulated IOLs were assayed for cytotoxic activity against ME 180 cells using MTT Mosmann, J. Immunol. Methods (1983) 65:65 to measure the amount of cell proliferation. Immunotoxin diluted in PBS alone or in polymer without drying was also evaluated using the method described above. The effects on cell proliferation (expressed as percent inhibition of cell growth) for a simulated IOL containing about 0.24 ug/ml immunotoxin are shown in Table 3.

The simulated IOLs were prepared by combining immunotoxins with any of a variety of polymers and then drying them onto tissue culture plates for three hours, as described above. The majority of immunotoxin polymer mixtures were as active as untreated immunotoxin in PBS in inhibiting cell proliferation. Hydroxpropylmethylcellulose and polyvinylpyrrolidone were slightly more active; these polymers alone exhibited some antiproliferative activity (percent inhibition of cell growth 17.3 and 21.6 for the two polymers, respectively). All other polymers alone had little or no antiproliferative activity. Immunotoxin mixed with Pluronic F127 and Pluronic F68 antiproliferative lost activity as a result of drying. Immunotoxin in these polymers without drying gave percent inhibitions of 87.8 and 91.1 respectively. Immunotoxin in all other polymers without drying had essentially the same activity as immunotoxin in PBS.

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Table 3

	Percent Inhibition of Cell Growth
Polymer[1]	Immunotoxin 4197X-RA in Polymer (dried 3 hours)[2]
Methylcellulose (medium viscosity) 2% (Sigma Chemical Company)	84
Carboxymethylcellulose (medium viscosity) 2% (Sigma Chemical Company)	83.1
Hydroxpropylmethylcellulose (low viscosity) 2% (Sigma Chemical Company)	88.7
Hydroxpropylmethylcellulose (high viscosity) 2% (Sigma Chemical Company)	100
Polyvinylpyrrolidone (PVP-360) 5% (Sigma Chemical Company)	100
Polyethylene Glycol 40% (Sigma Chemical Company)	76.2
Pluronic F127 20% BASF Corporation	42.8
Pluronic F68 20% BASF Corporation	43.9
Methocel E4M 1% Dow Chemical Company	86.7
Methocel E50LV 2% Dow Chemical Company	85.9
Methocel E5 5% Dow Chemical Company	82.6
Methocel E15LV 5% Dow Chemical Company	82.0
Polyvinyl alcohol 5% Aldrich Chemical Corp.	78.8

[1] The indicated % is the concentration of the polymer stock solution in PBS.

[2] 4197X-RA in PBS Control (not dried) resulted in 86.8% inhibition of cell growth.

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Example 7
Release of 4197X-RA
Immunotoxin from Intraocular Device Coated
Onto a Simulated IOL and Cytotoxicity

Polystyrene balls were used to simulate IOLs for this study. The immunotoxin used in these experiments was 4197X-RA. One part of immunotoxin was mixed with two parts Methocel E4M (1% in PBS) and one part maltose (1M). The release of radioactivity from immunotoxin-polymer coated polystyrene balls (1/4 inch diameter, Polysciences) over time is shown in Table 4. The release of cytotoxic activity from coated balls is shown in Table 5. Preparation of materials used in these experiments was performed with sterile reagents under sterile conditions.

In the experiment to determine rate of release of radioactivity from the simulated IOLs, the 4197X-RA was labeled with ^{125}iodine. Individual balls were dipped into the coating mixture and allowed to air dry for two hours at room temperature. The coated balls were then dipped into a 5% solution of Methocel E15LV and allowed to air dry for 2 hours. This step was repeated two more times. After the final dip in Methocel E15LV the coated balls were allowed to air dry at room temperature overnight. Individual immunotoxin coated balls were then immersed into 1 ml of PBS (for determination of radioactive release) or M199 containing 10% FBS (for determination of cytotoxic activity release). At specified times balls (3 per time period for radioactivity, 2 per period for cytotoxity) were removed from incubation solutions. Ball-associated radioactivity was determined by placing the balls in a gamma counter.

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Table 4
Release of ^{125}-I-4197X-RA from Intraocular Device (Polymer=Methocel E4M) Coated Onto A Simulated IOL Support

Time (Minutes)	Percent Released Radioactivity[1]
10	7.9
20	17.9
30	21.6
60	46.7
120	70.2

[1] Values are the mean of 3 determinations using 3 balls per time point.

Cytotoxic activity associated with the incubation solution was determined by titration of the solution and exposing the dilutions to ME 180 cells. An MTT assay was used to determine the percent inhibition of cellular proliferation.

Table 5
Release Of 4197X-RA Cytotoxicity From Intraocular Device (Polymer=Methocel E4M) Coated Onto A Simulated IOL Support

Time (Minutes)	Percent Cytotoxic Activity Released[1]
10	3
20	25
30	42
60	50
120	100

[1] Values are the mean of 2 determinations using 2 balls per time point.

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Example 8
Evaluation of Cytotoxicity of 3D4-RA Immunotoxin
Intraocular Device Coated Onto
an IOL Over Time

5

The release of cytotoxic activity of an immunotoxin (3D4-RA) from an intraocular device coated onto an IOL was evaluated. The following steps were performed with sterile reagents under

10 sterile conditions. Immunotoxin 3D4-RA (1.56 mg/ml) was mixed with Methocel E4M and maltose as described in Example 7. Four polymethylmethacrylate (PMMA) intraocular lenses (Alcon) were dipped into the mixture. Two of the IOLs were allowed to dry and

15 coated with Methocel E15LV as in Example 7 and the remaining two were immediately placed in 1 ml M199 containing 10% FBS and incubated for 6 hours at 4°C. After 24 hours the coated and dried IOLs were placed in M199 and incubated for 6 hours at 4°C?. Cytotoxic

20 activity of the extracts was analyzed on ME 180 cells using MTT to determine inhibition of cellular proliferation as described in Examples 6 and 7. The cytotoxic activity extracted from IOLs coated with 3D4-RA in polymer and dried for 24 hours was the same

25 as that extracted from IOLs coated but not dried (control).

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Example 9
In Vivo Analysis of 3D4-RA Immunotoxin[1] Intraocular
Device Coated IOLs for the Prevention of
Secondary Cataract

5

Immunotoxin associated with an IOL would place
the cytotoxic agent in immediate proximity with the
target cell population and increase residence time of
the immunotoxin and the cytotoxic agent in the eye.
10 These factors could lead to a more efficacious
killing of target lens epithelial cells. Secondary
cataract typically develops in rabbits within a few
weeks following cataract surgery as indicated by
proliferation of residual lens epithelial cells on
15 the posterior lens capsule. The effect of IOLs
coated with a 3D4-RA immunotoxin intraocular device
on proliferation of residual lens cells was evaluated
in vivo in a rabbit model. In this study, a 3D4-RA
immunotoxin device-coated IOL was used.
20   Four normal female white New Zealand rabbits
were randomly assigned to two treatment groups: (1)
control group, and (2) immunotoxin treatment group.
Both groups underwent lens extraction surgery with
phacoemulsification in one eye. The surgical
25 procedure simulated the method used on humans to
remove primary cataracts. Group 1 received untreated
lenses, group 2 received lenses treated with a
polymer coating containing immunotoxin as described
in Example 8.
30   All of the rabbits received one week of standard
post-surgical care involving anti-inflammatory
(topical steroid and antibiotic (topical gentamicin)

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[1] 4197X does not bind rabbit lens epithelial cells, an
immunotoxin was constructed using a different
35 monoclonal antibody, 3D4. This antibody, while
being less specific than 4197X, does bind rabbit
lens epithelial cells and kills these cells in vitro
when coupled to ricin A.

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treatment. The animals were observed for four weeks
after surgery. At four weeks all animals were
sacrificed and their eyes were collected. None of
the animals exhibited gross toxicological or
5   pathological reactivities to the implanted lenses.

Eyes were processed for histology, embedded into
parafin and cut into thin sections, individual
sections were stained and examined microscopically.
The microscopic evaluation of the explanted eye
10   sections revealed that the control eyes containing
the untreated lenses exhibited epithelial cell growth
on anterior and posterior walls of the lens capsule,
i.e., development of secondary cataract. Deeper
sections showed substantial epithelial cell piling on
15   the anterior wall with a single layer of epithelial
cells present on the posterior capsule wall with
additional piling of cells at the equatorial regions.

The examination of sections from eyes containing
the immunotoxin-treated lenses revealed little or no
20   cellular growth on the lens capsule except in the
extreme equatorial regions. Epithelial cell piling
on the anterior capsule and epithelial growth on the
posterior capsule were decreased absent. The
decreased amount of epithelial cell growth in the
25   immunotoxin-treated eyes was considered significant.
No evidence of toxicity in the iris, choroid, or
retina segments of the eye, was noted in any lens
type.

These preliminary results, even though conducted
30   with a small group of animals, are significant in
that they suggest (1) that an immunotoxin having the
capacity to bind to lens epithelial cells can prevent
development of secondary cataract, and (2) it is
possible to deliver immunotoxin in a polymeric
35   carrier via an IOL.

Coating IOLs with intraocular devices comprising
solutions of immunotoxin in 10% F68 provides a

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mechanism to deliver immunotoxin in association with
the IOL. Active (as determined by its ability to
kill target cells) immunotoxin is released into the
IOL environment over time. All of the IOL-associated
5   immunotoxin is released from the IOL within 24 hours.

By implanting the subject intraocular device
into the anterior chamber, remnant lens epithelial
cells can be prevented from proliferating, thus
10   avoiding secondary cataracts. The use of an
intraocular device either as a stand alone device or
with an IOL support provides a superior means for
delivery of immunotoxin by allowing for release of
immunotoxin over time and protection of the
15   immunotoxin from degradative processes in the eye.
The subject methods and device provide a simple
procedure for preventing secondary cataracts.

All publications and patent applications
20   mentioned in this specification are indicative of the
level of skill of those skilled in the art to which
this invention pertains. All publications and patent
applications are herein incorporated by reference to
the same extent as if each individual publication or
25   patent application was specifically and individually
indicated to be incorporated by reference.
The invention now having been fully described,
it will be apparent to one of ordinary skill in the
art that many changes and modifications can be made
30   thereto without departing from the spirit or scope of
the appended claims.

47.

WHAT IS CLAIMED IS:

1. An intraocular device comprising:
a biologically inert polymer and a
cytotoxic agent capable of binding at least
substantially specifically to epithelial cells,
wherein said cytotoxic agent is associated reversibly
with said polymer.

2. The device according to Claim 1, wherein
said cytotoxic agent is an immunotoxin.

3. The device according to Claim 2, wherein
said immunotoxin comprises an antibody at least
substantially specific for epithelial cells
conjugated to a toxic agent.

4. The device according to Claim 3, wherein
said antibody is a monoclonal antibody.

5. The device according to Claim 4, wherein
said toxic agent is a ricin A-chain.

6. The device according to Claim 1, wherein
said polymer is coated onto the surface of an
intraocular lens or an "O" ring of a form suitable
for use with an intraocular lens.

7. The device according to Claim 6, wherein
said "O" ring is degradable in the eye.

8. A method for preventing secondary cataracts
which form following extracapsular extraction, said
method comprising:
implanting, during or immediately after
extracapsular extraction, an intraocular device into
the anterior or posterior chamber of the eye, wherein

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said intraocular d vice comprises a biologically
inert polymer and a cytotoxic agent capable of
binding at least substantially specifically to
epithelial cells, and wherein said cytotoxic agent is
5   associated reversibly with said polymer and when
released from said polymer is present in the anterior
chamber for a time and in an amount sufficient to
kill at least substantially all residual lens
epithelial cells.
10

        9.   A method for preparing an intraocular
device, said method comprising:
        contacting a biologically inert polymer with a
cytotoxic agent capable of binding at least
15   substantially specifically to epithelial cells, so
that said cytotoxic agent is associated reversibly
with said polymer.

        10.   The method according to Claim 9, wherein
20   said associated reversibly is entrapment of said
cytotoxic agent within said polymer or adsorption of
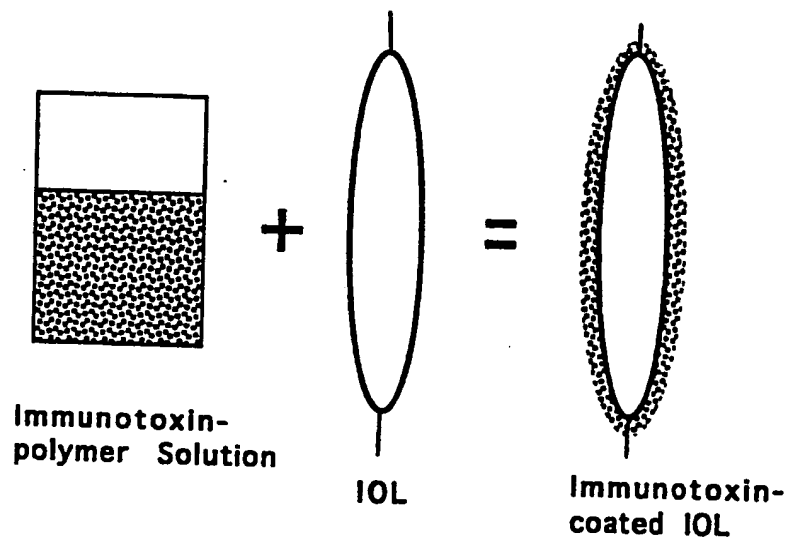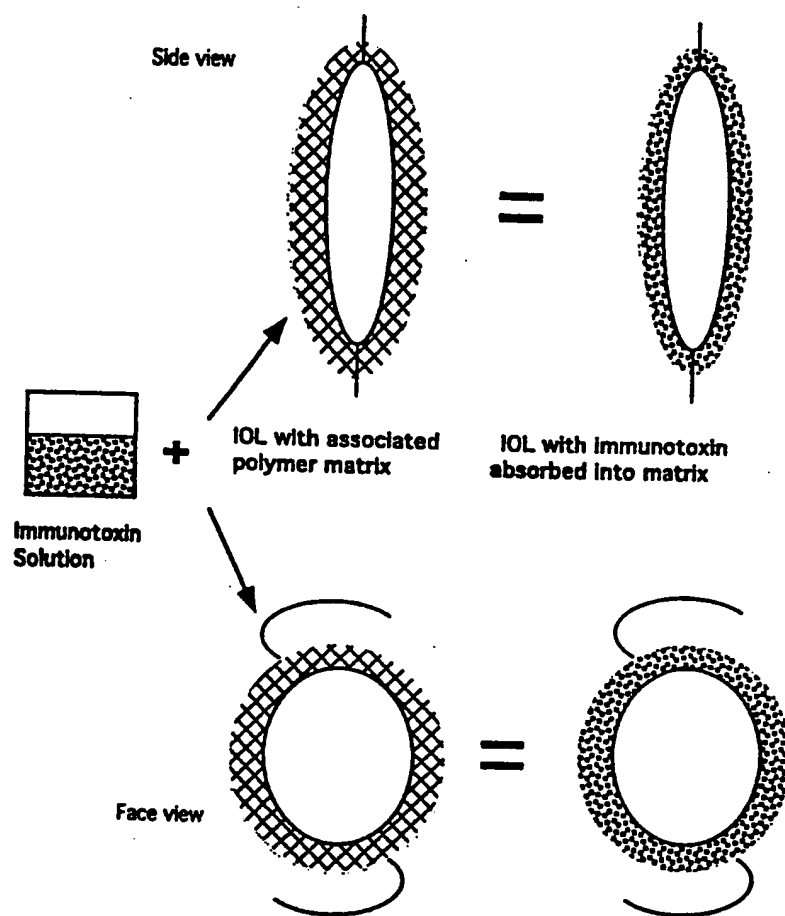said cytotoxic agent to said polymer.

1/3

Immunotoxin-
polymer Solution   +   IOL   =   Immunotoxin-
                                              coated IOL

FIGURE 1

SUBSTITUTE SHEET

Side view

Immunotoxin
Solution

+

IOL with associated
polymer matrix

IOL with immunotoxin
absorbed into matrix

Face view

FIGURE 2

SUBSTITUTE SHEET
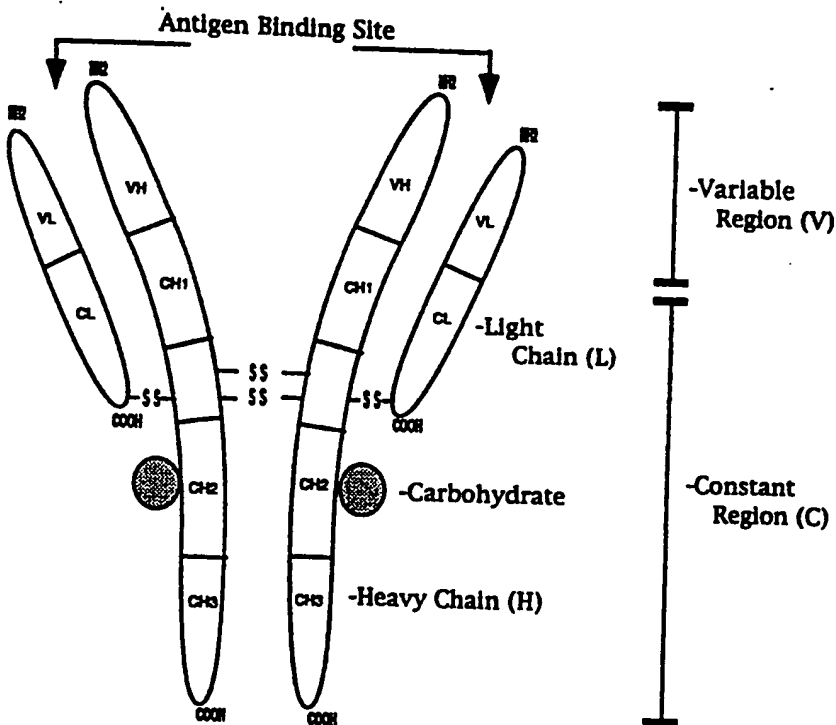
FIGURE 3

INTERNATIONAL SEARCH REPORT

| Box I | Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet) |

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

REMARK: Although claim 8 is directed to a method of treatment of the human body the search has been carried out and based on the alleged effects of the composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

| Box II | Observations where unity of invention is lacking (Continuation of item 2 of first sheet) |

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1992)

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0299467	18-01-89	US-A-	4918165	17-04-90
		AU-B-	602993	01-11-90
		AU-A-	1904388	19-01-89
		JP-A-	1100133	18-04-89
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